EPA/OPP MICROBIOLOGY LABORATORY
ESC, Ft. Meade, MD

Standard Operating Procedure
for
Sterility Assessment of Disinfectant Product Samples

SOP Number: QC-18-03

Date Revised: 07-12-05

Initiated By: _____ Date: ___/___/___

Print Name:_____

Technical Review: _____ Date: ___/___/___

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QA Officer

Approved By: _____ Date: ___/___/___

Print Name:_____
Branch Chief

Effective Date: ___/___/___

Controlled Copy No.: _____

Withdrawn By: _____ Date: ___/___/___

TABLE OF CONTENTS

1.0 SCOPE AND APPLICATION:

1.1 This protocol describes quality control practices that may be performed on disinfectant product samples to assess their sterility.

2.0 DEFINITIONS: None

3.0 HEALTH AND SAFETY:

3.1 Disinfectants may contain a number of different active ingredients, such as heavy metals, aldehydes, peroxides, phenol, etc. Latex gloves and other personal protective clothing or devices are worn during the handling of these items. A chemical fume hood or other containment equipment is employed when performing tasks with contain products.

4.0 CAUTIONS: None

5.0 INTERFERENCES:

5.1 Aseptic procedures must be followed during this assay to avoid accidental contamination of the product. Exposing the product to external contaminants during opening and dispensing, and the use of non-sterile laboratory supplies may interfere with the outcome of this analysis. Quality control measures for media, reagents and pre-sterilized supplies used in this evaluation must be followed as outlined in SOP QC-11, Performance and Sterility of Media and Reagents.

5.2 Cloudiness of media tubes due to the interaction of the disinfectant and medium may interfere with the evaluation of the media tubes. A Gram stain is performed on the cloudy media to verify the presence or absence of microbial growth.

6.0 PERSONNEL QUALIFICATIONS:

6.1 Personnel are required to be knowledgeable about the procedures in this SOP. Documentation of training and familiarization with this SOP can be found in the training file for each employee.

7.0 SPECIAL APPARATUS AND MATERIALS:

7.1 Incubator with temperature reading at the appropriate temperatures, $37 \pm 1^{\circ}C$ and $55 \pm 1^{\circ}C$

7.2 VITEK® 32 Identification System

8.0 INSTRUMENT OR METHOD CALIBRATION:

8.1 Refer to the laboratory equipment calibration and maintenance SOPs (SOP EQ series) for details on method and frequency of calibration.

9.0 SAMPLE HANDLING AND STORAGE:

9.1 Disinfectants are stored according to manufacturers' recommendations or at room temperature if the product label or testing parameters do not identify a storage temperature.

10.0 PROCEDURE AND ANALYSIS:

10.1 General Guidelines:

10.1.1 Procedures such as opening the product container, preparing serial dilutions, and inoculating media must be performed under aseptic conditions in a biological safety cabinet.

10.1.2 The sterility assessment should be performed when the product container is initially opened.

10.1.3 Sterility assessments may be performed prior to or concurrently with an efficacy test.

10.1.4 Always follow appropriate chain of custody procedures as stipulated in SOP COC-01, Sample Login and Tracking.

10.1.5 A neutralizer recommended for the product's active ingredient(s) should be used as the diluent (see 10.4). Information on the appropriate neutralizer is included in the product's test parameter.

10.2 Preparation and Opening the Sample Container:

10.2.1 The container must be opened under aseptic conditions in a biological safety cabinet.

10.2.2 For liquids, prior to opening the container, gently shake the container and thoroughly clean the area around the cap and spout with 70% ethanol. Allow the surface to dry.

Remove the cap. Do not touch the inside surface of the cap. If a seal is present, carefully remove the seal attached to the lip of the spout with sterile instruments (i.e., razor blade, forceps).

10.2.3 For spray products, shake the container at least 25 times immediately prior to assay. Remove cap and clean the nozzle and wipe top of can with 70% alcohol. Allow the surface to dry. Don sterile gloves. Spray the product for 10-15 seconds prior to collection of sample.

10.2.4 For towelette products, clean the dispenser or packaging with 70% alcohol. Allow the surface to dry. Aseptically remove a towelette by wearing sterile latex gloves and with the use of sterile forceps.

10.3 Collection of the Sample:

10.3.1 For liquids, pour approximately 10 mL of the sample into a sterile beaker. Do not place a pipette or any other instrument inside the product container. Place cap on the product container and secure tightly. Initiate serial dilutions from this sample (see 10.4).

10.3.2 For a spray product, spray the product into a sterile beaker for 20-30 seconds. Allow the product to settle (i.e., run down the sides of the beaker). Approximately 10 mL of liquid should be collected by this method. Initiate serial dilutions from this sample (see 10.4). When collecting the sample (i.e., spraying), hold the spray can in one hand, perpendicular to the biological safety cabinet surface. Hold the beaker in the other hand, positioning it so that it is parallel to the biological safety cabinet surface with the open end facing the nozzle of the spray can. The potential for contamination of the sample (i.e., contact of product with sprayer's gloved hand) is reduced as compared to positioning the spray can directly over the beaker (on biological safety cabinet surface) and spraying down into the beaker.

10.3.3 For a towelette, if saturated, carefully express the liquid from a single towelette by squeezing out the liquid into a sterile beaker. Use sterile forceps to manipulate the towelette. Collect approximately 10 mL of the liquid. More than one towelette may be required to collect a 10 mL sample. Initiate serial dilutions

from this sample (see 10.4).

If the towelette is not saturated with liquid, carefully place a folded towelette into 20 mL of letheen broth (38 mm x 100 mm tube) or other suitable neutralizer. Gently agitate the tube containing the towelette. Carefully extract the towelette with sterile forceps. Express the liquid during the extraction. Initiate serial dilutions from the residual mixture remaining in the tube (see 10.4).

10.3.4 A preparation number must be given to the sample. Fill in information on a Media Preparation form as stipulated in SOP QC-15, Media Prep and Sterilization Run Numbers. In addition, fill in appropriate information on the Sterility Assessment of Product Sample Record (see 16.1).

10.4 Preparation of Serial Dilutions:

10.4.1 Prepare the first dilution by pipetting 1 mL of the sample into a 9 mL tube of diluent. Prepare dilutions of 1×10^{-1} through 1×10^{-5}. Vortex each dilution tube prior to a transfer. Include a tube of undiluted sample (approx. 5 mL in a 20 mm x 150 mm tube) in the dilution set.

10.5 Inoculation of Culture Media:

10.5.1 Label the media tubes to correspond with the appropriate dilution. Inoculate 10 mL tubes (20 mm x 150 mm) of letheen broth and fluid thioglycollate medium in duplicate with 1 mL of each dilution; include the undiluted sample as well.

10.5.2 Include one tube of letheen broth and fluid thioglycollate medium as uninoculated controls. Thus, a total of 26 tubes (13 letheen broth, 13 fluid thioglycollate medium) will be inoculated per sample.

10.5.3 Incubate tubes at $37 \pm 1^{\circ}C$ for at least 48 hours. Record (on the Sterility Assessment of Product Sample Record) the time that tubes are placed in the incubator. Proceed with section 10.6.

10.5.4 Once results have been read and recorded following incubation at $37 \pm 1^{\circ}C$, incubate tubes at $55 \pm 1^{\circ}C$ for at least 48 hours. Record

(on the Sterility Assessment of Product Sample Record) the time that tubes are placed in the incubator. Proceed with section 10.6 again.

10.5.4.1 Tubes which are positive (i.e., have growth) following incubation at 37±1°C are not incubated at 55±1°C. Store the tubes in the refrigerator. Record "NA" for "Not Applicable" in the results blocks of the Sterility Assessment of Product Sample Record for tubes not incubated at 55±1°C. Include a footnote indicating that tubes were refrigerated rather than incubated at 55±1°C.

10.6 Results and Confirmation:

10.6.1 Record (on the Sterility Assessment of Product Sample Record; next to "Date Recorded/Initials") the time that results are read.

10.6.2 Each tube is shaken prior to recording results to determine the presence or absence of turbidity. Report results as + (growth) or 0 (no growth) on the Sterility Assessment of Product Sample Record (see 16.1). A positive result is one in which microbial growth is observed. A negative result is one in which the broth appears clear.

10.6.2.1 If a tube exhibits cloudiness due to the presence of the disinfectant, record the observation as "NR" (=not readable) on the form. Additionally, perform a Gram stain on the tube to verify the presence or absence of microbial growth. Record Gram stain results on the Worksheet for Recording Gram Stain and Acid Fast Reactions (see 16.2). It is not necessary to repeat staining of "NR" tubes stained following incubation at 37±1°C unless tubes appear different (i.e., more turbid) following incubation at 55±1°C. If cells are observed, note this in the comments section of the Sterility Assessment of Product Sample Record. From the tube, streak the culture on a plate of TSA for initial isolation. Attempt to identify the contaminant by using the VITEK® 32 system (see SOP QC-16, VITEK: Culture Identification Numbers).

10.6.3 Growth from at least one representative positive tube (showing

turbidity) from each medium will be Gram stained and streaked on TSA for initial identification and isolation. Record Gram stain results on the Worksheet for Recording Gram Stain and Acid Fast Reactions (see 16.2). Attempt to identify the contaminant by using VITEK® system. Record confirmation results on the Test Microbe Confirmation Sheet (see 16.3).

11.0 DATA ANALYSIS/CALCULATIONS: None

12.0 DATA MANAGEMENT/RECORDS MANAGEMENT:

12.1 Data will be recorded promptly, legibly, and in indelible ink on the appropriate forms. Completed forms are archived in notebooks kept in secured file cabinets in the file room D217. Only authorized personnel have access to the secured files. Archived data is subject to OPP's official retention schedule contained in SOP ADM-03, Records and Archives.

13.0 QUALITY CONTROL:

13.1 For quality control purposes, the required information is documented on the appropriate record form(s) (see 16.0).

14.0 NONCONFORMANCE AND CORRECTIVE ACTION:

14.1 No further product testing will be initiated if product contamination is detected.

15.0 REFERENCES: None

16.0 FORMS AND DATA SHEETS:

16.1 Sterility Assessment of Product Sample Record

16.2 Worksheet for Recording Gram Stain and Acid Fast Reactions

16.3 Test Microbe Confirmation Sheet

Sterility Assessment of Product Sample Record

OPP Microbiology Laboratory

PRODUCT INFORMATION		BACKGROUND and PREPARATION NUMBERS	
Confirmed by:_____		Confirmed by:_____	
EPA Reg. No.		Date performed/initials	
Name		Letheen broth prep. No.	
Sample No.		Fluid thioglycollate prep. No.	
Lot No.		Other prep No.:	
Container No.			

RESULTS: Date Recorded/Initials:_____

Dilution of Sample	Letheen Broth (+/0)*				Fluid Thioglycollate Medium (+/0)*			
	Tube 1 @ 37°C	Tube 1 @ 55°C	Tube 2 @ 37°C	Tube 2 @ 55°C	Tube 1 @ 37°C	Tube 1 @ 55°C	Tube 2 @ 37°C	Tube 2 @ 55°C
Undiluted Sample								
10^{-1}								
10^{-2}								
10^{-3}								
10^{-4}								
10^{-5}								
Uninoculated Media Control			NA	NA			NA	NA

* + = growth, 0 = no growth
NR=Not Readable
NA=Not Applicable

COMMENTS**

** diluent, tubes selected for confirmation, growth characteristics, additional dates results recorded

Worksheet for Recording Gram Stain and Acid Fast Reactions

OPP Microbiology Laboratory

Slide ID:__________ Test:____________ _________________ Product:__________ _________________ Date:_____________ Initials:	Source/Tube No. _________________ Results:___________	Source/Tube No. _________________ Results:___________	Source/Tube No. _________________ Results:___________

Slide ID:__________ Test:____________ _________________ Product:__________ _________________ Date:_____________ Initials:	Source/Tube No. _________________ Results:___________	Source/Tube No. _________________ Results:___________	Source/Tube No. _________________ Results:___________

Slide ID:__________ Test:____________ _________________ Product:__________ _________________ Date:_____________ Initials:	Source/Tube No. _________________ Results:___________	Source/Tube No. _________________ Results:___________	Source/Tube No. _________________ Results:___________

AF+ =Acid fast positive
GPC =Gram positive cocci
GNR =Gram negative rod
GPR = Gram positive rod

Test Microbe Confirmation Sheet
OPP Microbiology Laboratory

TEST INFORMATION/ Confirmed by:_____			
EPA Reg. No.		Test Date	
Name		Test Organism	
Sample No.		Comments:	

Source: Tube/Plate ID	Date/ Initials	Stain Results*	Media Information			Results		
			Type	Prep. No.	Inc. Time/ Temp.	Date/ Initials	Colony Characteristics	API Test**/VITEK ID (if applicable)

* Record Acid Fast or Gram Stain results as GPC=gram positive cocci, GNR=gram negative rods, AFR=acid fast rods, GPR=Gram positive rods.
** API numerical profile number